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ABSTRACT OF DISCLOSURE

The present invention relates to isolated polypeptide products encoded by the LIPG gene and analogues, fragments, derivatives, or mutants thereof which are derived from such polypeptides and which retain at least one biological property thereof (hereafter, collectively, "LIPG polypeptide"). The present invention relates also to compositions and methods for regulating the levels of HDL cholesterol and apolipoprotein AI, VLDL cholesterol and LDL cholesterol. The compositions and methods of the present invention function by raising or lowering the levels or enzymatic activity of LIPG polypeptide.--

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REMARKS

Reconsideration of the allowability of the present application in view of the above amendments and the following remarks is requested respectfully.

Status of the Claims

Claims 1, 7, 8, 10, 11, 13 to 16, 19 to 23, 40, 41, 43 to 47, 49 to 53, 55 to 59, and 63 to 95 were pending at the time of Examiner's Action. Claims 1, 7, 8, 10, 11, 13 to 16, 19, 21 to 23, 40, 41, 43 to 47, 49 to 53, 55 to 59, 63 to 65, and 73 have been withdrawn as being drawn to a non-elected invention.

With the present Amendment, Claims 20, 85, 90, and 91 have been amended and Claims 66 to 70, 72, 77, and 92 have been canceled without prejudice. Claims 96 and 97 have been added. The pending elected claims are Claims 20, 71, 74 to 76, 78 to 91, and 93 to 97.

Discussion of the Amendments

Support for the amendment to Claim 20 is found at pages 25, 26, and 83 and Example 7 of the specification as filed originally. Claim 20 has been amended also to incorporate the recitation of Claim 77, now canceled. Support for the amendment to Claim 85 is found at Example 7 of the specification. Support for the amendments to Claims 90 and 91 is found at page 26 and page 29, last two full paragraphs, of the specification as filed originally. Support for Claims 96 and 97 is found at pages 26 and 27 of the specification as filed originally. Amendments of an editorial nature were made to the descriptive portion of the specification and to the abstract.

Discussion of Examiner's § 102(b) Rejection of Claims 20, 69 to 71, and 74 to 84

The Examiner has rejected composition Claims 20, 69 to 71, and 74 to 84 as being anticipated by Cooper et al., *Biochim. Biophys. Acta* (1989), 1008:92-101. Claims 20, 69 to 71, and 74 to 76 were rejected alternatively as being anticipated by Cooper et al., GenBank Database, Accession No. P 11602 (1989). According to the Examiner, each of the Cooper et al. publications (hereafter, collectively "Cooper et al.") discloses a lipoprotein lipase which has a sequence which is about 50% identical to SEQ ID NO: 10. The Examiner's rejection with respect to Claim 69 and 70 have been rendered moot by the cancellation of those claims.

Applicants submit respectfully that the Examiner's rejection has been overcome by the present amendment to independent Claim 20, from which Claims 71 and 74 to 84 depend. For a reference to anticipate a claim, the reference must teach each and every element of the claimed invention. MPEP §706.02. Independent Claim 20 recites that the composition comprises a polypeptide which comprises a 19-residue lid region which is characteristic of LIPG polypeptide. As stated in the application at page 26, the lid region is the region with the greatest sequence divergence in the

triacylglycerol lipase family and the LIPG polypeptide lid region is three residues shorter than the corresponding lid region of lipoprotein lipase. Cooper et al. does not disclose a polypeptide with such a lid region. Accordingly, Cooper et al. does not disclose the polypeptide which is defined by applicants' composition claims.

Traversal of the Examiner's § 102(b) Rejection of Claim 95

The Examiner has rejected Claim 95 as being anticipated by Cooper et al., *Biochim. Biophys. Acta* (1989), 1008:92-101, as evidenced by Gershenwald et al., *Biochim. Biophys. Acta* (1985), Abstract, 836:286-95. According to the Examiner, Cooper et al. teaches an avian lipoprotein lipase in which an 18-mer fragment is identical to a segment of SEQ ID NO: 10. According to the Examiner, this 18-mer fragment is large enough to induce an immune response.

Applicants traverse respectfully the Examiner's rejection. For a reference to anticipate a claim, the reference must teach each and every element of the claimed invention. MPEP §706.02. Claim 95 recites an antigenic fragment of a polypeptide which is encoded by the LIPG gene. Cooper et al. does not disclose an antigenic fragment of LIPG polypeptide. While Cooper et al. may disclose a polypeptide that has some similarities to LIPG polypeptide, there is no disclosure in Cooper et al. that any fragment of such a polypeptide may have antigenic properties. In fact, Cooper et al. does not disclose any fragment at all.

Discussion of the Examiner's Rejection of Claims 20, 66 to 72, 77 to 85, 88, 92, and 95 under the Written Description Requirement of §112, First Paragraph

The Examiner's rejection of Claims 20, 71, 77 to 85, 88, and 95 under the written description requirement of §112, first paragraph, is traversed respectfully. The Examiner's rejection of Claims 66 to 70, 72, and 95 has been rendered moot by the

cancellation of those claims.

According to the Examiner, "[t]o fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these." Applicants note that not one but at least two species of LIPG polypeptide have been fully described (SEQ ID NOS.: 6 and 8). In contrast to what the Examiner has said, SEQ ID NO.: 6 is not a truncated version of SEQ ID NO.: 8 as residues 346 to 354 of SEQ ID NO.: 6 are not the same as the corresponding residues of SEQ ID NO.: 8. Applicants have described also various features common to all LIPG polypeptides. Among these are: (A) a 39kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; and (B) a unique 19-residue lid region (the sequence between two framing cysteine residues in the sequence indicated by a bold line in Figure 6). SEQ ID NOS.: 6 and 8, both forms of LIPG polypeptide, contain both of these features, with the 39 kD catalytic region present at residues 1 to 345 of both sequences and the lid region present at residues 253 to 271 of both sequences. The 39 kD catalytic region contains the conserved catalytic triad of the triacylglycerol lipase family, described in the paragraph bridging pages 6 and 7 of the specification. This region provides the polypeptide with lipase activity. The 19-residue lid region is unique to LIPG polypeptide and confers sequence specificity to the polypeptide, as described on page 26 of the specification as filed. Accordingly, applicants have described at least two species of LIPG with enough detail to permit one of skill in the art to predict the structure of other species encompassed by the

claimed genus. Applicants have described also structural characteristics common to all LIPG polypeptides and their correlation with their respective functions.

Discussion of the Examiner's Rejection of Claims 74 to 76
under the Written Description Requirement of §112, First Paragraph

The Examiner's rejection of Claims 74 to 76 under the written description requirement of §112, first paragraph, has been overcome by the above amendment to Claim 20, from which Claims 74 to 76 depend. According to the Examiner, Claims 74 to 76 are drawn to a polypeptide without definite structure. Claims 74 to 76 have been amended to include the recitation that the LIPG polypeptide recited therein: (A) binds heparin; (B) has homology with human lipoprotein lipase and hepatic lipase; (C) comprises a 39kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; (D) comprises a 19-residue lid region which is characteristic of LIPG polypeptide; and (E) has lipase activity.

Traversal of the Examiner's Rejection of Claim 87
under the Written Description Requirement of §112, First Paragraph

The Examiner's rejection of Claim 87 under the written description requirement of §112, first paragraph, is traversed respectfully.

According to the Examiner, Claim 87 is directed to a polypeptide but does not provide any definite structure of that polypeptide. Applicants note, however, that Claim 87 is dependent from Claim 85 which recites that the polypeptide: (A) binds heparin; (B) has homology with human lipoprotein lipase and hepatic lipase; (C) comprises a 39kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; (D) comprises a 19-residue lid region which is characteristic of

LIPG polypeptide; and (E) has lipase activity.

Discussion of the Examiner's Rejection of Claims 66 to 68
under the Written Description Requirement of §112, First Paragraph

The Examiner's rejection of Claims 66 to 68 under the written description requirement of §112, first paragraph, has been rendered moot by the cancellation of these claims.

Discussion of the Examiner's Rejections under §112, Second Paragraph

The Examiner's rejection of Claims 20, 71, and 74 to 84 as being indefinite in the use of the phrase "LIPG polypeptide" is traversed respectfully. The Examienr has noted that the abbreviation "LIPG" is undefined. The term "LIPG" is not an abbreviation but a technical term which is known by those of skill in the art to refer to the gene encoding endothelial lipase. Similarly, "LIPC" is the technical term for the gene encoding hepatic lipase. In addition, applicants note that the above claims have been amended to recite that the polypeptide is characterized as having: (A) a 39kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; and (B) a unique 19-residue lid region.

The Examiner's rejection of Claims 85 to 91 and 95 as being indefinite in the use of the phrase "LIPG polypeptide" has been overcome by the present amendment in which independent Claim 85, which Claims 86 to 91 and 95 contain the recitations of, has been amended to more definitively define the LIPG polypeptide.

The Examiner's rejection of Claims 85 to 91 and 95 as being indefinite because the phrase "the LIPG gene" implies the existence of only one such gene has been

rendered moot by the present amendment to Claim 85, from which Claims 86 to 91 depend and Claim 95 includes the recitations thereof. Nevertheless, applicants submit respectfully for the record that there is no ambiguity in the use of the term "LIPG gene". According to the Examiner, there exist various different LIPG polypeptides and, therefore, it is unclear as to why there exists only one such LIPG gene. Applicants note, however, that the expression of any given gene may produce various different polypeptides. For example, there may be splice variants or the mRNA produced from such a gene may be produced differently. In the present situation, the LIPG gene gives rise to both LLGN and LLGXL polypeptides.

The Examiner's rejection of Claims 85, 87 to 91, and 95 as being indefinite because the phrase "39 kD catalytic domain of the triacylglycerol lipase family" is unclear is traversed respectfully. Such a domain is defined in the specification. As described in the paragraph bridging pages 35 and 36 of the specification, an example of this catalytic domain is SEQ ID NO.: 10. As noted on page 30 of the specification, any analogue, fragment, derivative, or mutant which is derived from this sequence and which retains at least one biological property thereof is covered also by this definition. However, as the claims now recite, such analogues, fragments, derivatives, or mutants include a 19-residue lid region and a catalytic triad comprising serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274. Accordingly, the characteristics of the 39 kD catalytic domain are well defined and the claim language is clear.

The Examiner's rejection of Claims 90 and 91 as being indefinite for each referring to an amino acid sequence of SEQ ID NO: 8 has been overcome by the above amendment in which both Claims 90 and 91 have been amended to recite that the amino acid sequence comprises a sequence of SEQ ID NO: 8. As stated in the application at page 26, lines 20 to 23, the 68 kD polypeptide is likely a form of the 55

kD polypeptide.

The Examiner's rejections of Claims 66 to 70, 72, and 92 have been rendered moot by the cancellation of such claims.

Discussion of the Examiner's Objections

The Examiner's various objections to Claims 20, 66 to 72, 77 to 85, 87, and 92 have been addressed by the above amendments to the claims and to the descriptive portion of the specification.

With respect to the Examiner's rejection of Claim 95, it is noted that Claim 95 does indeed further define the subject matter of Claim 85 since Claim 95 recites that the fragment has antigenic activity.

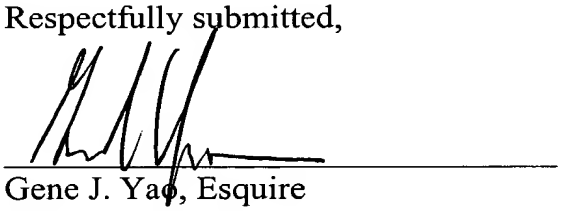
Conclusion

For the reasons expressed above, applicants request respectfully that the Examiner reconsider and withdraw her rejections under §§102 and 112 and her objections to the claims and the descriptive portion of the specification.

Attached hereto is a marked-up version of the changes made to the application by the current amendment. The attached version is captioned "Version with Markings to Show Changes Made."

In view of the foregoing amendment and remarks, an early and favorable action is requested respectfully.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Description

The paragraph commencing at page 21, line 17, has been amended as follows.

Figure 6 shows a protein sequence alignment of the members of the triacylglycerol lipase gene family (SEQ ID Nos: 13-15). Shaded residues are identical to the LLGXL protein (SEQ ID NO: 8). The deduced amino acid sequence of human LIPG(EL; endothelial lipase) (SEQ ID NO: 8) is provided on the top line and is compared with the other major members of the TG lipase family, LPL (lipoprotein lipase) (SEQ ID NO: 13), HL (heptaic lipase) (SEQ ID NO: 14) and PL (pancreatic lipase) (SEQ ID NO: 15). EL residues identical to those in at least one other member of the family are shaded as well as the corresponding residue in the other family member. Amino acids are numbered according to convention beginning with the initial residue of the secreted protein. The predicted sites of signal peptide cleavage are marked with a solid line between amino acid residues. The GXSXG lipase motif containing the active serine is boxed. The amino acids of the catalytic triad are marked with an asterisk. The conserved cysteines are marked with filled circles. Potential N-linked glycosylation sites are marked with arrowheads. The lid region is indicated by a bold line. Gaps were introduced into the sequences to maximize the alignment values using the CLUSTAL program.

The paragraph commencing at page 22, line 23, has been amended as follows.

Figure 10 shows the sequence of the immunizing peptide (SEQ ID NO: 16) and its relation to the LLGXL protein sequence (SEQ ID NO: 8). The peptide is shown in the shaded box. The terminal cysteine was introduced to aid coupling of the peptide to

the carrier protein.

The paragraph commencing at page 23, line 13, has been amended as follows.

Figure 13 shows the sequence of the rabbit LIPG PCR product (RLLG.SEQ, SEQ ID NO: 12) and the sequence alignment between the rabbit LIPG PCR product and the corresponding sequence in the human cDNA (LLG7742A) (SEQ ID NO: 7). Identical nucleotides are shaded.

The paragraph commencing at page 96, line 10, has been amended as follows.

A commercially available lambda cDNA library derived from rabbit lung tissue (Clontech, Cat. #TL1010b) was used to isolate a fragment of the rabbit homolog of the LIPG gene. Five microliters of the stock library were added to 45 ml water and heated to 95°C for 10 minutes. The following were added in a final volume of 100 ml: 200 mM dNTPs, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 100 mM each primer DLIP774 and LLGgen2a, and 2.5 U Taq polymerase (GIBCO). The reaction was thermocycled 35 times with the parameters of: 15 seconds at 94°C, 20 seconds at 50°C and 30 seconds at 72°C. Ten microliters of the reaction [was] were analyzed via agarose gel electrophoresis. A product of approximately 300 basepairs was detected. A portion (4 ml) of the reaction mix was used to clone the product via the TA cloning system. The insert of a resulting clone was sequenced (SEQ ID NO: 11). An alignment between the deduced rabbit amino acid sequence (SEQ ID NO: 12) and the corresponding sequence of the human cDNA is also shown in Figure [14] 13. Of the nucleotides not part of either amplification primer, there is an 85.8% identity between the rabbit and human LLG sequences. The predicted protein encoded by this rabbit cDNA shares a 94.6 % identity with that of the human protein, with most of the nucleotide substitutions in the third or "wobble" positions of the codons. Notably, this

region spans the "lid" sequence of the predicted LLG proteins and is a variable domain in the lipase gene family. This is evidence that there is a high degree of conservation of this gene between species.

The paragraph commencing at page 103, line 25, has been amended as follows.

To perform the experiments discussed in Examples 12 to 16, the following procedure (based on the procedure outlined in Example 1) was used to obtain the cDNA for LIPG. THP-1 cells were plated in the presence of phorbol 12-myristate 13-acetate (PMA, 40 ng/ml; Sigma) for 48 hours. The differentiated THP-1 cells were exposed for 24 hours to either oxLDL (50 μg/ml) or control medium. Total RNAs were collected and purified using standard procedures. Poly(A)$^+$ RNA was purified from total RNA using a poly-dT magnetic bead system (Promega). cDNA synthesis and PCR amplification were accomplished using protocols from the Differential Display kit, version 1.0 (Display Systems Biotechnology). The primer pairs that yielded the initial cDNA fragment of EL were downstream primer 7 (5'-TTTTTTTTTTTGA-3') (SEQ ID NO: 17) and upstream primer 15 (5'-GATCCAATCGC-3') (SEQ ID NO: 18). The amplification reaction was fractionated on a 6% nondenaturing acrylamide sequencing format gel and an amplification product found only in the reaction containing cDNA from THP-1 cells exposed to oxLDL was identified and excised from the gel. A reamplification using the same primers was performed and the product was excised and subcloned into the pCRII vector using the TA cloning system (Invitrogen). Insert sizes were determined using *EcoR*I digestions of the plasmids, and clones containing inserts of the approximate size of the original PCR product were sequenced using fluorescent dye-terminator reagents (Prism, Applied Biosystems) and an Applied Biosystems 373 DNA sequencer. We extended the cDNA sequence of the original, gel-excised cDNA using the 5'-RACE system (GIBCO). RNA (1 μg) from the THP-1 cells used initially in the

differential display reactions was used in the 5'-RACE procedure using a gene-specific primer (5'-TAGGACATGCACAGTGTAATCTG-3') (SEQ ID NO: 19) for first strand cDNA synthesis. We performed PCR amplification of the cDNA using an anchor primer and gene-specific primer 2 (5'-GATTGTGCTGGCCACTTCTC-3') (SEQ ID NO: 20). This reaction (1 μl) was used in a nested re-amplification using the universal amplification primer (5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3') (SEQ ID NO: 22) and the gene-specific primer 3 (5'-GACACTCCAGGGACTGAAG-3') (SEQ ID NO: 21) to increase levels of specific product for subsequent isolation. The reaction products were cloned into the pCRII vector from the TA cloning kit and [determined] the sequence determined. A human placental cDNA library (oligo dT and random primed) was obtained from Clontech and probed with the 5'-RACE reaction PCR product. The DNA from hybridizing clones was purified using LambdaSorb reagent (Promega). Inserts were excised from the phage DNA by digestion with *EcoR*I, subcloned into the *EcoR*I site of the Bluescript II SK plasmid vector (Stratagene), and sequenced.

The paragraph commencing at page 105, line 17, has been amended as follows.

A 17-residue peptide (GPEGRLEDKLHKPKATC) (SEQ ID NO: 16) was synthesized corresponding to residues 8-23 of the secreted LIPG gene product on a Model 433A peptide synthesizer (Applied Biosystems). Peptide (2 mg) was coupled to maleimide-activated keyhole limpet haemocyanin (2 mg) following the protocols included in the Imject Activated Immunogen Conjugation kit (Pierce Chemical). After desalting, one-half of the conjugate was emulsified with an equal volume of Freund's complete adjuvant (Pierce) and injected into a New Zealand White rabbit. Four weeks after the initial inoculation, a booster inoculation was administered with an emulsification made exactly as described above except for the use of Freund's incomplete adjuvant (Pierce). Two weeks after the boost, the titres of specific

antibodies were determined in a test bleed via ELISA using immobilized peptide.

The paragraph commencing at page 106, line 6, has been amended as follows.

HUVECs were propagated in a commercially prepared endothelial cell growth medium (EGM, Clonetics) supplemented with bovine brain extract (3 mg/ml; Clonetics), whereas HCAECs were propagated in EGM with bovine grain extract (3 mg/ml) and 5% fetal bovine serum. Cultures were stimulated by addition of PMA (100 ng/ml). After 24 hours incubation, RNA was extracted from the cells via the Trizol method, electrophoresed on a 1% agarose-formaldehyde gel, transferred to Nytran membrane on a Turboblotter apparatus (Schleicher and Schuell) and crosslinked to the membrane using a Stratalinker ultraviolet crosslinker (Stratagene). The 5'-RACE reaction PCR product was radiolabelled using the random priming technique. The radiolabelled probe ($1\text{-}2\times10^6$ cpm/ml) was denatured by heating to 95 °C for 10 minutes and quick-chilled on ice before adding to the filter in QuikHyb. Hybridization was allowed to proceed for 3 hours at 65 °C. Filters were exposed to Kodak XAR-2 film with intensifying screens at -80 °C. We incubated HUVEC- and HCEAC-conditioned medium with heparin-Sepharose CL-6B at 4 °C for 1 hour. After centrifugation, the pelleted heparin-Sepharose was suspended in SDS loading buffer, heated to 95 °C for 5 minutes and loaded onto a 10% Tris-Glycine SDS gel (NOVEX). After electrophoresis at 140 V for 90 minutes, the proteins were transferred to nitrocellulose membranes and detected with rabbit anti-LIPG peptide antisera (1:5,000), with goat anti-rabbit peroxidase conjugated antisera (1:5,000; Boehringer) as the secondary antibody. The membranes were reacted with Renaissance chemiluminescent reagent (DuPont NEN) and exposed to Kodak XAR-2 film. A commercially prepared filter containing poly(A)$^+$ RNAs (3 μg each) from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Clontech)was hybridized with a radiolabelled fragment and processed as described

above. Following autoradiography, the blot was stripped by washing in boiling 0.1xSSC, 0.1% SDS for 2x15 minutes at 65 °C and then probed as described above with a 1.4-kb cDNA fragment encoding human LPL. This fragment was obtained by RT-PCR of the THP-1 RNA (PMA and oxLDL treated) using the 5' LPL and 3' LPL primers 5'-ACCACCATGGAGAGCAAAGCCCTG-3' (SEQ ID NO: 24) and 5'-CCAGTTTCAGCCTGACTTCTTATTC-3' (SEQ ID NO: 25), respectively. After exposure to film, the membranes were stripped again and reprobed with a radiolabelled fragment of human β actin cDNA to normalize to RNA content.

In the Claims

20. (Amended three times) A composition for increasing the level of LIPG polypeptide in a patient comprising a pharmaceutically acceptable carrier and an LIPG polypeptide which: (A) binds heparin; (B) has homology with human lipoprotein lipase and hepatic lipase; (C) comprises a 39 kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; (D) comprises a 19-residue lid region which is characteristic of LIPG polypeptide; and (E) has lipase activity.

85. (Amended) An isolated LIPG polypeptide which [is encoded by the LIPG gene and which]: (A) binds heparin; (B) has homology with human lipoprotein lipase and hepatic lipase; [and] (C) comprises a 39 kD catalytic domain of the triacylglycerol lipase family which contains serine at residue position 169, aspartate at residue position 193, and histidine at residue position 274; (D) comprises a 19-residue lid region which is characteristic of LIPG polypeptide; and (E) has lipase activity.

90. (Amended) The polypeptide of Claim 85, wherein said polypeptide [has] comprises an amino acid sequence of SEQ ID NO: 8 and has an apparent molecular weight of about 55 kD on a 10% SDS-PAGE gel.

91. (Amended) The polypeptide of Claim 85, wherein said polypeptide [has] comprises an amino acid sequence of SEQ ID NO: 8 and has an apparent molecular weight of about 68 kD on a 10% SDS-PAGE gel.

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